CHROMOSOMES OF CONOCEPHALUM CONICUM

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(WITH PLATES IV, V)

The discovery of visible chromosome differences between the sexes in many animals has led to a very wide acceptance of the hypothesis that sex in animals is determined by the presence or absence of certain chromosomes. This discovery has stimulated botanists also to search for sex determinants, and experimental work has apparently demonstrated that in *Sphaerocarpos* and *Thallocarpus* two of the four spores formed by the division of a spore mother cell produce male plants and the other two female plants. In several dioecious mosses also experimental results indicate that the sex potentialities are probably separated in the reduction divisions. As yet, however, a visible chromosome difference between the sexes has been found in only two species of plants, *Sphaerocarpos Donnellii* reported by ALLEN,[1] and *S. texanus* reported by Miss SCHACKE.[2]

The present study of the chromosomes of *Conocephalum conicum* (L.) Dum. was begun primarily for the purpose of determining whether or not there exists a visible difference between the chromosomes of the two sexes in this species. The results in regard to this question are totally negative, but the chromosome number is found to be nine instead of eight, as reported by previous workers for the gametophytes of this species.

The material used in the greater part of the study was grown in the greenhouse in two separate cultures of male and female plants respectively. These cultures were started with thalli bearing old gametophores of the previous season's growth, collected

[1] ALLEN, C. E., A chromosome difference correlated with sex difference in *Sphaerocarpos*. Science, N. S. **46**:466–467. 1917.

———, The basis of sex inheritance in *Sphaerocarpos*. Proc. Amer. Phil. Soc. **58**:289–316. 1919.

[2] SCHACKE, M. A., A chromosome difference between the sexes of *Sphaerocarpos texanus*. Science, N. S. **49**:218. 1919.

November 8, 1919, in Parfrey's Glen near Merrimac, Wisconsin. The archegoniophores were removed to prevent the possibility of the development of male sporelings from the sporogones, which latter were well developed at that time. The plants grew rapidly, and apical tips of the plants of the two sexes were fixed at several different times during the latter half of December. A few almost mature antheridiophores fixed in the field and imbedded in paraffin were obtained from Dr. W. N. STEIL. In addition to this material Dr. ALFRED GUNDERSEN and Professor A. F. BLAKESLEE generously supplied living plants from stock received from Copenhagen, and Professor A. J. EAMES sent plants from Cascadilla Ravine, Ithaca, New York. Comparative studies were made on these plants, but all figures shown were drawn from the Wisconsin material.

Flemming's medium solution with 4 per cent of urea added was used in fixing. Paraffin sections 4–6 μ thick in the case of the apical tips and 3 μ thick in the case of the antherids were stained with Flemming's triple combination. The sections on a few slides were restained in Heidenhain's haematoxylin, but gave results less satisfactory than those obtained with the triple stain.

The resting nuclei and stages in the formation of the spirem were not examined critically in this study. Numerous nuclei in spirem stages and in equatorial plate stages were found in these preparations, but very few cases of spirem segmented into chromosomes not yet drawn into the equatorial zone of the spindle were seen. Evidently, as observed by FARMER,[3] the transition from the unsegmented spirem stage to the equatorial plate stage is very rapid, if indeed the migration toward the equatorial region does not begin before the segmentation of the spirem, as evidenced by the frequently observed tendency of the chromosomes to lie end to end in the equatorial plate (figs. 2, 3, 9). The limited number of observations, however, does not justify any conclusion on this point.

Judging by the large number of nuclei in the equatorial plate stage, a considerable pause in the movement of the chromosomes occurs at this point, which also coincides with FARMER'S observations. The longitudinal splitting of the chromosomes does not

[3] FARMER, J. B., On spore formation and nuclear division in the Hepaticae. Ann. Botany **9**:469–523. 1895.

become apparent until very late; in fact, it is perceptible only when the separation of the daughter chromosomes has actually begun (figs. 13–15). The chromosomes are in the form of bent, crinkly rods of varying lengths (figs. 1–11). The crinkliness is less apparent in the late metaphases and in the anaphases when the chromosomes are drawn out into smooth rods (figs. 12, 16, 19). As observed by ESCOYEZ,[4] they occupy a very definite plane in the equatorial plate stage; in polar view they are easily counted at this time, but in lateral view they appear as tangled masses (figs. 8, 14, 15). Only one case (fig. 11) was found of an equatorial plate stage in which the individual chromosomes could be traced with any degree of certainty in a lateral view, and a very few such cases in anaphases (figs. 12, 16, 19). The chromosome number is plainly nine in either sex (figs. 1, 2, 4, 9, and 18 female; figs. 3, 5, 6, 8, and 10 male), one of the chromosomes being very small. This small chromosome shows no constant difference in behavior from the other chromosomes, either as to its position on the spindle or in its time of division. In one case (fig. 15) it was found to have been divided earlier than the other chromosomes, and in another case it was found undivided in the equator of the spindles when the other chromosomes were in anaphase (fig. 19). Metaphases and anaphases in which the individual chromosomes are distinguishable are very rarely found, but if the small chromosome constantly led the way in division, as it appears to do in fig. 15, or if it constantly lagged, as seems to be the case in fig. 19, it should usually be visible in the metaphases and anaphases, even though the other chromosomes are not distinguishable one from another. Apparently the small chromosome ordinarily divides at about the same time as the other chromosomes, and in lateral view is distinguishable from them only in rare cases (figs. 12, 15, 16).

In cells of the apical tip of the thallus (of either sex) in which the chromosomes are commonly spread out so as to make accurate counts possible, the small chromosome is visible in about 80 per cent of the cases counted; but in the antherid, where the cells are much smaller and the chromosomes generally more closely grouped,

[4] ESCOYEZ, E., Blepharoplaste et centrosome dans le *Marchantia polymorpha*. La Cellule **24**:247–256. 1907.

it is visible in a much smaller percentage of the cases. Considering the size of this chromosome, it is to be expected that in some cases it should be obscured from vision by the other chromosomes (figs. 7, 8, 14, 17).

FARMER, BOLLETER,[5] ESCOYEZ, and WOODBURN[6] report eight chromosomes in the haploid nucleus, and in my preliminary note[7] I suggested the possibility of a variation as to chromosome number in this species. More recent studies in plants from Ithaca and from Copenhagen make it seem quite certain that the same number of chromosomes is to be found in the plants of this species in those regions. It seems probable, therefore, that these investigators have overlooked the small chromosome, a thing which might easily have happened, especially since they were interested primarily in other phenomena.

A comparison of the chromosomes of one sex with those of the other shows no perceptible difference, either in the number or size relations, as may be seen by comparing figs. 1, 2, 4, 7, 9, and 18 (female) with figs. 3, 5, 6, 8, and 10 (male). Although this condition of like chromosomes in the two sexes in *Conocephalum* is not an evidence against the sex chromosome basis of sex inheritance in the dioecious Bryophyta, it does show that the marked difference between the chromosomes of the two sexes in *Sphaerocarpos* is not a universal condition among these plants.

Summary

1. The chromosome number in the gametophyte of *Conocephalum conicum* (L.) Dum. is nine instead of eight as reported by previous investigators.

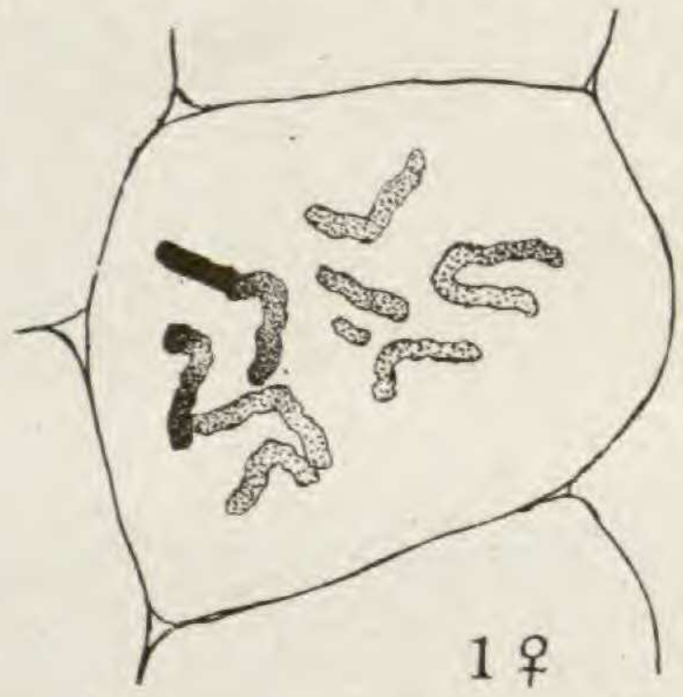
2. The chromosomes vary considerably in size, one being very much smaller than any of the other eight.

3. There is no perceptible difference between the chromosomes of the male and those of the female plant.

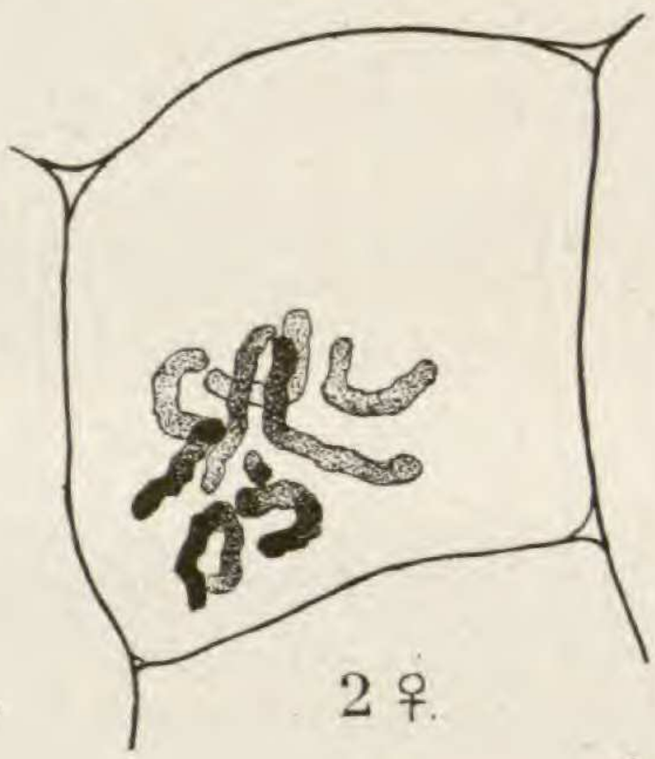
[5] BOLLETER, E., *Fegatella conica*, eine morphologisch-physiologische Monographie. Beih. Bot. Centralbl. **18**:327–408. 1905.

[6] WOODBURN, W. L., Spermatogenesis in certain Hepaticae. Ann. Botany **25**:299–313. 1911.

[7] SHOWALTER, A. M., The chromosomes of *Concephalum conicum*. Science, N. S. **53**:333. 1921.



1 ♀



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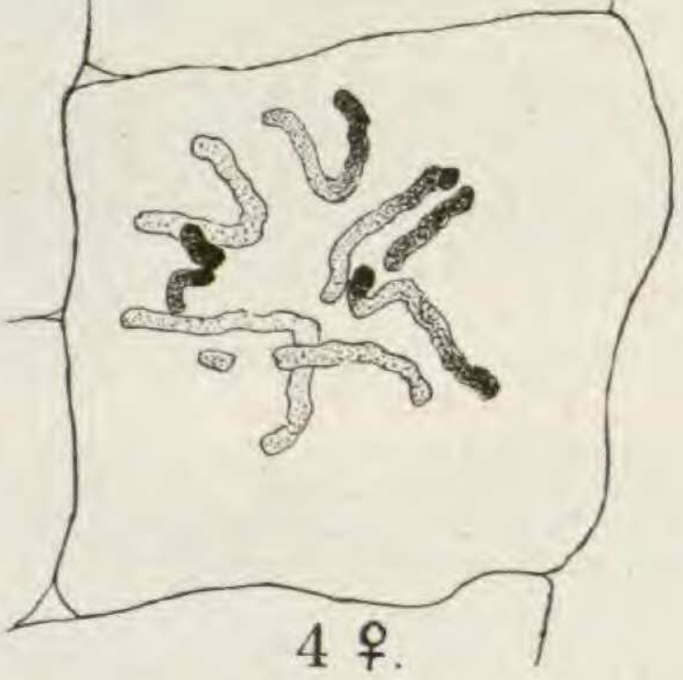

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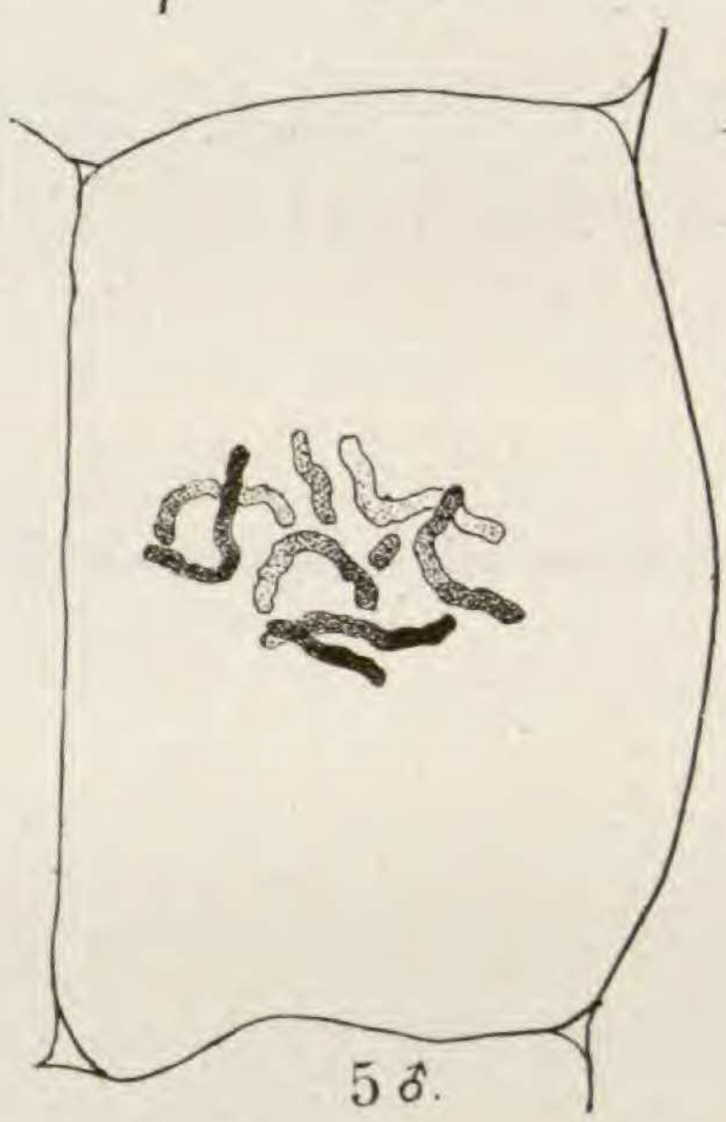

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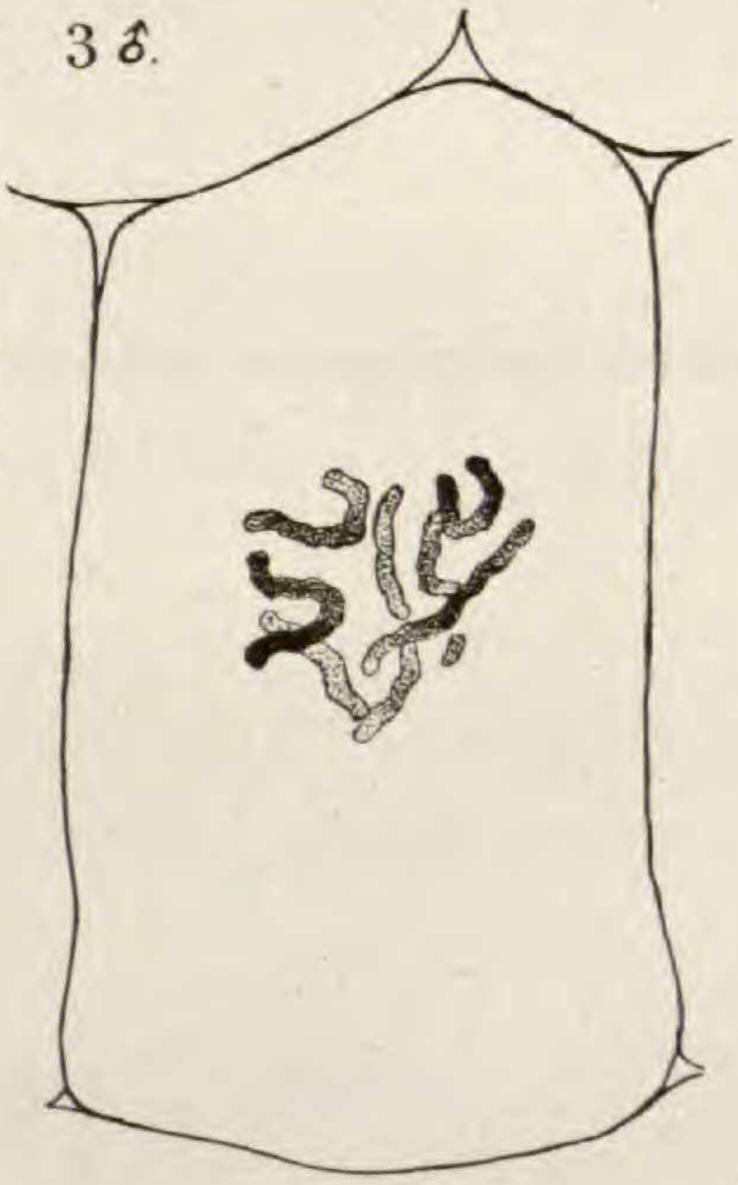

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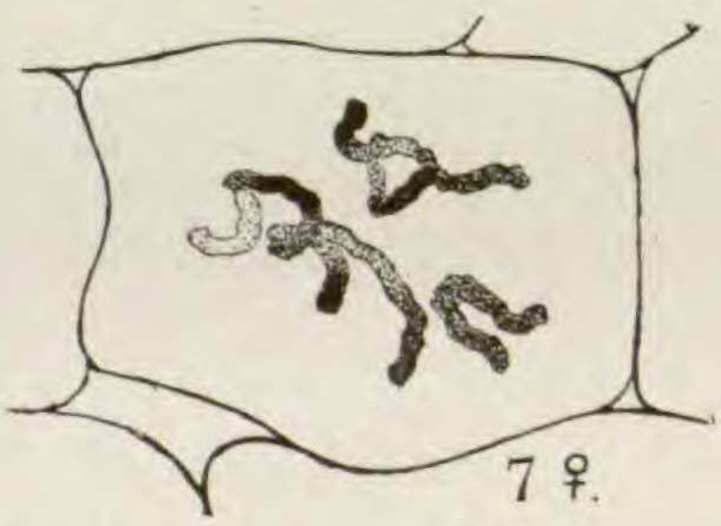
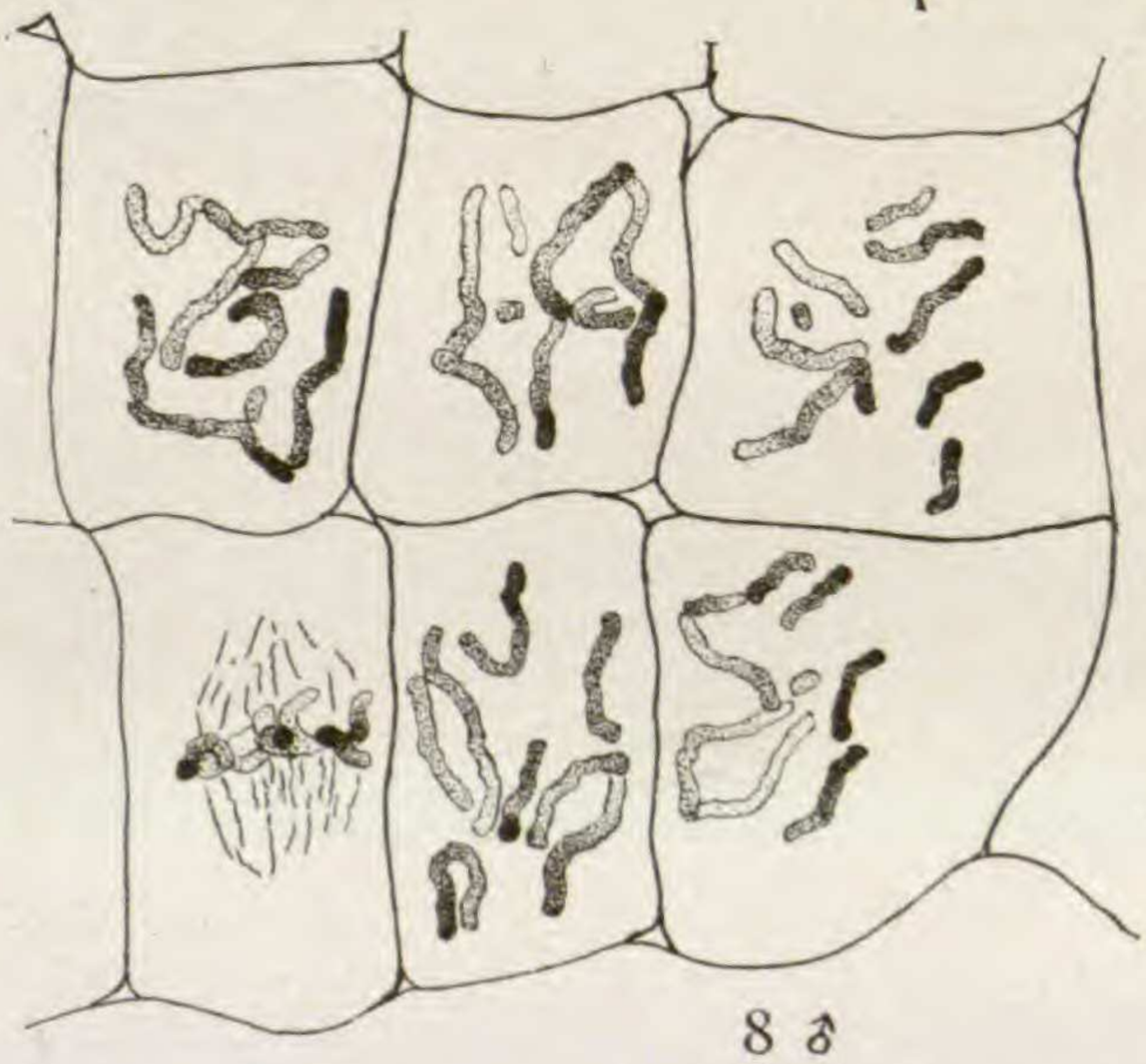
9 ♀.



7 ♀.



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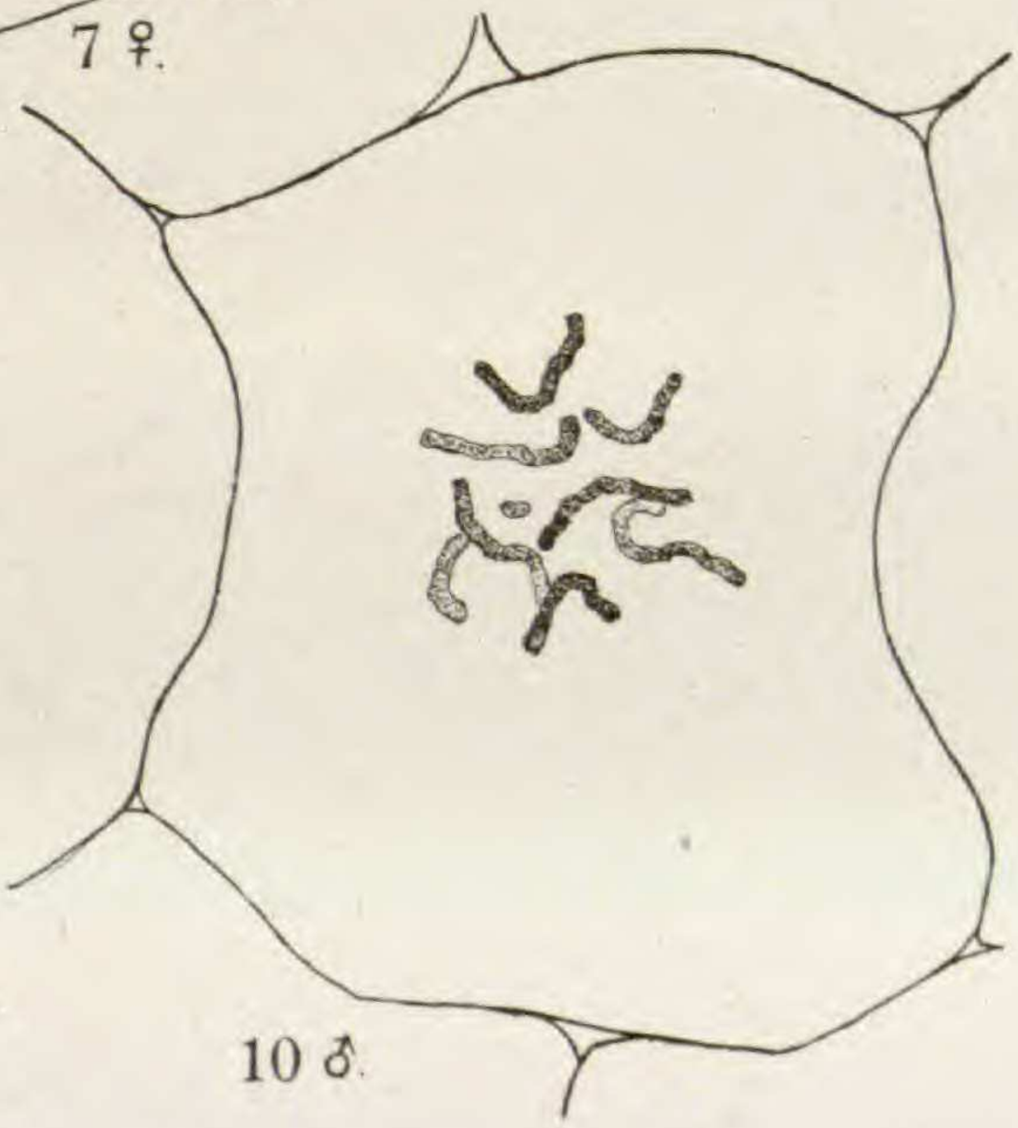

10 ♂.

SHOWALTER on CONOCEPHALUM